

CLAIMS

I claim:

1. A method of isolating and amplifying a nucleic acid molecule, the method comprising

(a) ligating a nucleic acid molecule into a linear vector to form a circular vector comprising the vector and the nucleic acid molecule,

wherein the linear vector is a double-stranded linear nucleic acid comprising two nucleic acid strands, wherein the second strand of the circular vector is discontinuous, and wherein the first strand in the circular vector is a closed circular strand,

(b) amplifying the first strand by rolling circle replication to form tandem sequence DNA,

wherein the amplification results in amplification of the nucleic acid molecule in the first strand.

2. The method of claim 1 wherein the second strand of the linear vector contains at least one nick, wherein the nick cannot be ligated.

3. The method of claim 1 wherein either the 5' or the 3' end of the second strand of the linear vector cannot be ligated.

4. The method of claim 1 wherein the second strand of the linear vector contains at least one gap or overlap.

5. The method of claim 1 wherein the method further comprises, following ligation and prior to amplification, separating the first strand from the second strand.

6. The method of claim 5 wherein the second strand includes an affinity tag.

7. The method of claim 6 wherein the first strand is separated from the second strand by binding the affinity tag to a substrate, denaturing the first and second strands prior to, simultaneous with, or following binding, and separating the first strand from the substrate.

8. The method of claim 5 wherein the second strand of the linear vector contains at least one overlap, part of the overlapping portions of the second strand

are complementary, and the 3' end of the overlap extends beyond the part of the overlapping portions that are complementary,

wherein the first strand is separated from the second strand by ligating one end of the second strand to a nucleic acid molecule coupled to a substrate, denaturing the first and second strands following ligation of the second strand, and separating the first strand from the substrate.

9. The method of claim 1 wherein step (a) comprises ligating a plurality of nucleic acid molecules into a plurality of linear vectors in a single reaction to form a plurality of circular vectors, each circular vector containing at least one nick, gap, or overlap in the second strand,

wherein step (b) comprises amplifying the first strand of the plurality of circular vectors, and

wherein the method further comprises, prior to amplification, dividing the ligation reaction to produce a plurality of separate amplification reactions.

10. The method of claim 9 further comprising making a replica of the amplification reactions.

11. The method of claim 10 wherein the replica of the amplification reactions is made by contacting the amplification reactions with a surface to which nucleic acids can bind.

12. The method of claim 10 wherein the replica of the amplification reactions is made by transferring part of each amplification reaction to form a replica amplification reaction.

13. The method of claim 9 wherein the ligation reaction is divided by spreading the ligation reaction onto a surface to form a spread, and wherein the separate amplification reactions are the locations of circular vectors on the surface after spreading.

14. The method of claim 13 further comprising making a replica of the amplification reactions.

15. The method of claim 14 wherein the replica is made by contacting the spread with a second surface to which nucleic acids can bind.

16. The method of claim 9 wherein any number or all of the amplification reactions are ordered as an array of reaction droplets or in an array of reaction vessels.

17. The method of claim 16 wherein, following amplification, all or part of the contents of any number or all the individual reaction droplets or reaction vessels are transferred by one to one mapping to a new set of reaction droplets or reaction vessels.

18. The method of claim 17 further comprising, following amplification, determining the presence of amplified nucleic acid in the amplification reactions, and

transferring all or a part of the contents of the amplification reactions containing amplified nucleic acid reaction to a new set of reaction droplets or reaction vessels.

19. The method of claim 16 further comprising making a replica of the amplification reactions.

20. The method of claim 19 wherein the replica of the amplification reactions is made by contacting the amplification reactions with a surface to which nucleic acids can bind.

21. The method of claim 19 wherein the replica of the amplification reactions is made by contacting the amplification reactions with a surface treated with an affinity target capable of binding an affinity tag, wherein the amplified nucleic acid comprises affinity tags incorporated during amplification,

wherein a portion of each amplification reaction is transferred to the surface.

22. The method of claim 21 wherein the affinity tag is biotin and the affinity target is streptavidin.

23. The method of claim 21 wherein the affinity tag is a reactive moiety and the affinity target is a corresponding reactive moiety, where a chemical reaction between the affinity tag and the affinity target results in the amplified nucleic acid being covalently coupled to the surface.

24. The method of claim 23 wherein the affinity target is phenylene diisothiocyanate, disuccinimidylcarbonate, disuccinimidylloxolane or dimethylsuberimidate and the affinity tag is a reactive amine.

25. The method of claim 19 wherein the replica of the amplification reactions is made by transferring part of each amplification reaction to form a replica amplification reaction.

26. The method of claim 16 wherein, following amplification, all or part of the contents of any number or all of the reaction droplets or reaction vessels are transferred and combined to create one or more sets of pooled reactions.

27. The method of claim 16 wherein the amplification reactions are arranged on the surface of a substrate.

28. The method of claim 27 wherein the substrate comprises acrylamide, cellulose, nitrocellulose, polystyrene, polyethylene vinyl acetate, polypropylene, polymethacrylate, polyethylene, polyethylene oxide, glass, polysilicates, polycarbonates, teflon, fluorocarbons, nylon, silicon rubber, polyanhydrides, polyglycolic acid, polylactic acid, polyorthoesters, polypropylfumerate, collagen, glycosaminoglycans, polyamino acids, chemical resistant metals, or corrosion resistant metals.

29. The method of claim 9 wherein the method further comprises, prior to dividing the ligation reaction,

diluting the ligation reaction such that, on average, each amplification reaction contains a single circular vector.

30. The method of claim 9 wherein the method further comprises, following amplification, collecting a sample of each amplification reaction.

31. The method of claim 9 wherein the method further comprises detecting or sequencing the nucleic acid molecules in the amplification reactions or in the collected samples.

32. The method of claim 1 wherein rolling circle replication is primed by the second strand.

33. The method of claim 1 wherein rolling circle replication is primed by a rolling circle replication primer.

34. The method of claim 33 wherein the tandem sequence DNA is amplified by strand displacement replication to form secondary tandem sequence DNA.

35. The method of claim 34 wherein the secondary tandem sequence DNA is amplified by strand displacement replication to form tertiary tandem sequence DNA.

36. The method of claim 34 wherein strand displacement replication of the tandem sequence is primed by a strand displacement primer.

37. The method of claim 9 further comprising detecting one or more amplified nucleic acid molecules in one or more of the amplification reactions.

38. The method of claim 37 wherein the nucleic acid molecules are derived from cDNA generated by suppression subtractive hybridization.

39. The method of claim 37 wherein the plurality of nucleic acid molecules are all derived from the same source.

40. The method of claim 37 further comprising, following amplification, creating a replica of the amplification reactions, contacting the amplification reactions with a first set of labeled nucleic acid probes and the replica amplification reactions with a second set of labeled nucleic acid probes, and

comparing the pattern of hybridization of the first set of probes to the pattern of hybridization of the second set of probes,

wherein differences in the patterns of hybridization indicate differences in the probe sets.

41. The method of claim 40 further comprising selecting for isolation or further analysis amplification reactions that hybridize to the first set of probes but not to the second set of probes, amplification reactions that hybridize to the second set of probes but not to the first set of probes, amplification reactions that hybridize to the both sets of probes, or amplification reactions that do not hybridize to either set of probes.

42. An *in vitro* method of cloning nucleic acid molecules, the method comprising

- (a) dividing a nucleic acid sample to produce a plurality of separate amplification reactions,
- (b) amplifying nucleic acid molecules in the amplification reactions,
- (c) making a replica of the amplification reactions,
- (d) testing nucleic acid molecules in either the amplification reactions or the replica amplification reactions to identify nucleic acid molecules of interest, and
- (e) retrieving the identified nucleic acid molecules of interest from the corresponding amplification reactions or replica amplification reactions that were not tested.

43. The method of claim 42 wherein the nucleic acid sample is divided by spreading the sample onto a surface to form a spread, and wherein the separate amplification reactions are the locations of circular vectors on the surface after spreading.

44. The method of claim 43 wherein the replica of the amplification reactions is made by contacting the spread with a second surface to which nucleic acids can bind.

45. The method of claim 42 wherein the method further comprises, prior to dividing the nucleic acid sample,
diluting the nucleic acid sample such that, on average, each amplification reaction contains a single nucleic acid molecule.

46. A method of isolating and amplifying nucleic acid molecules, the method comprising

- (a) ligating a plurality of nucleic acid molecules into a plurality of linear vectors in a single reaction to form a plurality of circular vectors, each circular vector comprising a vector and a nucleic acid molecule,

wherein the linear vectors are double-stranded linear nucleic acid comprising two nucleic acid strands, wherein the circular vectors each contain at least one nick,

gap, or overlap in the second strand, and wherein the first strand in each circular vector is a closed circular strand,

- (b) separating the first strands from the second strands,
- (c) diluting and dividing the first strands to produce a plurality of separate amplification reactions that, on average, each contain a single circular vector,
- (d) amplifying the first strands of the plurality of circular vectors by rolling circle replication to form tandem sequence DNA,

wherein the amplification results in amplification of the nucleic acid molecules in the first strands.

47. The method of claim 46 wherein the tandem sequence DNA is amplified by strand displacement replication to form secondary tandem sequence DNA.

48. The method of claim 47 wherein the secondary tandem sequence DNA is amplified by strand displacement replication to form tertiary tandem sequence DNA.

49. A method of isolating and amplifying a nucleic acid molecule, the method comprising

- (a) ligating a nucleic acid molecule into a linear vector to form a circular vector comprising the vector and the nucleic acid molecule,

wherein the linear vector is a double-stranded linear nucleic acid comprising two nucleic acid strands, wherein the circular vector contains at least one nick in the second strand and wherein the first strand in the circular vector is a closed circular strand,

- (b) amplifying the first strand,

wherein the amplification results in amplification of the nucleic acid molecule in the first strand.

50. A kit for isolating and amplifying nucleic acid molecules, the kit comprising

- (a) a linear vector wherein the linear vector is a double-stranded linear nucleic acid comprising two nucleic acid strands, and wherein

- (1) the linear vector contains at least one nick, wherein the nick cannot be ligated,

(2) either the 5' or the 3' end of the second strand of the linear vector cannot be ligated,

(3) the second strand of the linear vector contains at least one gap,

(4) the second strand of the linear vector contains at least one overlap, or

(5) any combination of (1), (2), (3) or (4);

(b) a rolling circle replication primer, wherein the rolling circle replication primer is complementary to a portion of the first strand of the linear vector; and

(c) a strand displacement primer, wherein the strand displacement primer matches a portion of the first strand of the linear vector.

51. A linear vector wherein the linear vector is a double-stranded linear nucleic acid comprising two nucleic acid strands, wherein the second strand of the linear vector contains an affinity tag, and wherein

(1) the linear vector contains at least one nick, wherein the nick cannot be ligated,

(2) either the 5' or the 3' end of the second strand of the linear vector cannot be ligated,

(3) the second strand of the linear vector contains at least one gap,

(4) the second strand of the linear vector contains at least one overlap, or

(5) any combination of (1), (2), (3) or (4).

52. A linear vector wherein the linear vector is a double-stranded linear nucleic acid comprising two nucleic acid strands, and wherein the second strand of the linear vector contains at least one overlap, part of the overlapping portions of the second strand are complementary, and the 3' end of the overlap extends beyond the part of the overlapping portions that are complementary.

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